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PATENT

Docket No. 1377-0137P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS: John Kevin Collins *et al.*

APPLN. NO.: 09/367,105

GROUP: 1651

FILED: November 10, 1999

EXAMINER: I. Marx

FOR:

PROBIOTIC STRAINS FROM
LACTOBACILLUS SALIVARIUS AND
ANTIMICROBIAL AGENTS OBTAINED
THEREFROM

DECLARATION UNDER 37 C.F.R. § 1.132

Assistant Commissioner of Patents

Washington, DC 20231

Sir:

I, John Kevin Collins, am presently employed as Vice President for Research and as Associate Professor in the Departments of Medicine and Microbiology, at University College Cork - National University of Ireland, Cork, College Road, Cork, Ireland. My *Curriculum Vitae* is attached hereto. I do solemnly and sincerely declare as follows:

1. I am authorized to make this Declaration on behalf of the Applicants.
2. I am an inventor in respect of the above Application and I attended at an interview with the Examiner on January 17, 2003.

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During the interview, which was also attended by Mr. Eugene Perez and Dr. MaryAnne Armstrong of the firm Birch Stewart Kolach & Birch LLP, the invention was discussed and it was agreed that amended claims would be filed and these were filed by way of a Supplemental Amendment on January 22, 2003.

3. As requested by the Examiner, I have given further consideration to certain definitions queried by her, namely the terms "mutant", "variant" and "closely related bacteria" as used in the context of the present invention.
4. As regards a definition of a variant/mutant, it was agreed at the interview that variants were inclusive of mutants. In addition I stated that variants could include extra-chromosomal genetic elements, e.g. plasmids, transposons, bacteriophage, which would genotypically and phenotypically add new properties to a strain without mutation or changing the base sequence of the bacterial genome. As I recall it was agreed after a detailed discussion at the interview that the term "variant" was the most comprehensive and suitable definition currently available.
5. As regards the definition of "closely related bacteria", I would refer to previous submissions filed during the course of prosecution of this Application. I would also stress that bacteriocins have classically been described as secreted proteinaceous factors that inhibit similar strains to that of the strains produced or other closely related strains. In this context,

6. I also wish to comment on the matter of the selection of adherent strains as discussed with the Examiner Dr. Marx during the interview. As I advised the Examiner we are in possession of a suite of lactobacillus strains selected from washed gastrointestinal tissue. *In vitro* adhesion assays illustrate that all of these strains adhere to human gastrointestinal epithelial cell lines (Caco-2 and HT-29). This is in contrast to strains isolated from other environments, e.g. dairy products. Direct comparisons with commercially available strains, the majority of which are faecal in origin, show that the strains isolated from the human gut have superior adherence properties. While we have isolated a suite of strains in this way, for resource reasons

we have focused our research on two strains, namely UCC1 and UCC118.

7. I also advised the Examiner that in subsequent research I have screened thousands of faecal lactobacillus isolates from a number of volunteers and have failed to find strains with similar antimicrobial profiles of UCC1 and UCC118. This is further proof that isolation of strains from washed and resected tissue provides access to strains with specific properties. These confer a selective survival advantage to the strain within the human gastrointestinal tract. Further ongoing research is confirming these results. I also advised the Examiner that bacterial strains that colonise the human gut are specifically adapted (humanized) to a unique microenvironment which may be the result of thousands of years of co-evolution. This was illustrated in human feeding studies where UCC118 is the only strain known to colonize a human volunteer for greater than one hundred days following cessation of feeding. This unique finding supports the case that strains isolated in this manner (from resected and washed human gastrointestinal tract) have unique abilities to adhere, survive and compete in the complex milieu of microbes, mucus and mucosal immune system that is the human gastrointestinal tract.

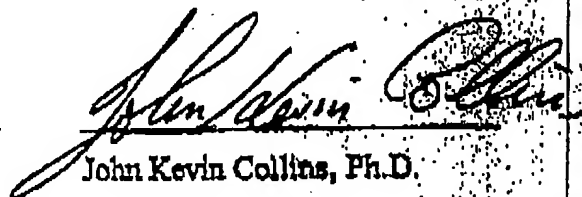
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I hereby declare that all statements made herein of my own knowledge are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

February 13, 2003

Date


John Kevin Collins, Ph.D.

NAME:

John Kevin Collins

POSITION:

Vice-President for Research, University College Cork
Associate Professor in Departments of Medicine & Microbiology
University College Cork

QUALIFICATIONS

1970 BSc (Biochemistry/Microbiology), University College Cork
1974 PhD (Biochemistry/Microbiology), National University of Ireland

Brief Outline of Academic History

1995-present Associate Professor, Depts. of Medicine & Microbiology, UCC
1992-1995 Associate Professor, Dept. of Microbiology, UCC
1983-1992 Senior Lecturer, Dept. of Microbiology, UCC
1977-1983 College Lecturer, Dept. of Microbiology, UCC
1982-1983 Visiting Research Professor, Bacteriology Department, University of California at Davis, USA
1976-1977 Post-Doctoral Research Fellow, Depts. of Pathology & Bacteriology, University of California at Davis, USA
1975-1976 NIH Post-Doctoral Research Fellow, Department of Microbiology, Health Science Center, State University of New York at Stony Brook, New York, USA
1974-1975 Damon Runyon Cancer Research Fund Post-Doctoral Fellow, Depts. of Pharmacology and Anatomy, Case Western Reserve University Medical School and Hospital, Cleveland, Ohio, USA
1970-1974 PhD Graduate Student, University College Cork.

RESEARCH INTERESTS

- ❖ Gastroenterology
- ❖ Gut Flora – including hitherto unculturable.
- ❖ Interaction of the gut flora with the mucosal immune system in health and disease.
- ❖ Inflammatory Bowel Disease – both Crohn's Disease and Ulcerative Colitis.
- ❖ Gastrointestinal Cancer.
- ❖ Probiotics as new therapeutic sources, e.g. from bugs to drugs.
- ❖ Taking probiotics from bench to bedside.
- ❖ Elucidating mechanisms of action of scientifically proven probiotic action.
- ❖ Virology – new antiviral agents.

Number of peer reviewed publications:

110+

Number of completed graduate student theses:

20 PhD; 18 MSc

Research funding generated:
(individually and with colleagues)

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Selected Research Publications:

- Flynn, S., van Sinderen, D., Thornton, G.M., Holo, H., Nes, I.F. & Collins, J.K.
Characterisation of the genetic locus responsible for the production of ABP-118, a novel bacteriocin produced by the probiotic bacterium *Lactobacillus salivarius* subsp. *salivarius* UCC118.
Microbiology, 2002. Apr; 148 (Pt. 4): 973-84.
- Fanning, L., Loane, J., Kenny-Walsh, E., Sheehan, M., Whelton, M., Kirwan, W., Collins J.K. & Shanahan, F.
Tissue viral load variability in chronic hepatitis C.
Am. J. Gastroenterol. 2001 Dec; 96(12): 2284-9.
- O'Sullivan, G., Ryan, P., Aarons, S., Walsh, T., Sheahan, D., Collins, J.K., & Shanahan, F. 2001.
Bone marrow micrometastasis in esophageal cancer : incidence and response to chemotherapy.
Recent Advances in Disease of the Esophagus 8: 385-388.
- Aarons, S., Ryan, P., O'Sullivan, G., Sheahan, D., Collins, J.K. & Shanahan, F. 2001.
Stable expression of transgenes in esophageal cancer : implication for development of gene therapy. *International Society for Diseases of the Esophagus* 8: 403-407.
- Bennett, M.W., O'Connell, J., Houston, A., Kelly, J., O'Sullivan, G.C., Collins, J.K. & Shanahan, F.
Fas ligand upregulation is an early event in colonic carcinogenesis.
J. Clin. Pathol. 2001. Aug; 54 (8): 598-604.
- O'Mahony, L., Feeney, M., O'Halloran, S., Murphy, L., Kiely, B., Fitzgibbon, J., Lee, G., O'Sullivan, G., Shanahan, F. & Collins, J.K.
Probiotic impact on microbial flora, inflammation and tumour development in IL-10 knockout mice.
Aliment Pharmacol Ther. 2001. Aug; 15(8): 1219-25.
- Barry, O.P., Mullan, B., Sheehan, D., Kazanietz, M.G., Shanahan, F., Collins, J.K. & O'Sullivan, G.C.
Constitutive ERK 1/2 activation in esophagogastric rib bone marrow micrometastatic cells is MEK-independent.
J. Biol. Chem. 2001 May 4; 276(18): 15537-46.
- Dunne, C., O'Mahony, L., Murphy, L., Thornton, G., Morrissey, D., O'Halloran, S., Feeney, M., Flynn, S., Fitzgerald, G., Daly, C., Kiely, B., O'Sullivan, G.C., Shanahan, F. & Collins, J.K.
In vitro selection criteria for probiotic bacteria of human origin : correlation with *in vivo* findings.
Am. J. Clin. Nutr. 2001 Feb; 73: 386-392.